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UHPLC-Q-TOF/MS detection of UV-induced TpT dimeric lesions in genomic DNA

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ABSTRACT

Ultraviolet (UV) radiation induces mutagenicity and cytotoxicity in human cells by the formation of DNA lesions, including cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs), mainly on thymine-thymine (TpT) dinucleotides. Here, we firstly synthesized the two TpT dimeric lesions with satisfactory yields using a unique UV-irradiated water droplet approach followed by HPLC purification. By the use of purified TpT lesions as standards, we further developed and optimized a quantitative UHPLC-Q-TOF/MS method for the detection of CPDs and 6-4PPs. After the optimization of the enzyme composition and the pH values of hydrolysis solution, a combination of snake venom phosphodiesterase, nuclease P1, and calf intestine alkaline phosphatase can be used for one-step enzymatic digestion to efficiently release the dimeric lesions (CPDs and 6-4PPs) from the genomic DNA. By the use of the one-step digestion and UHPLC-Q-TOF/MS assay for scanning all dimeric lesions, we demonstrate that only are TpT dimeric lesions detectable in genomic DNA of HCT116 cells upon UVC irradiation. The estimated frequency of the CPD of TpT increases from 28.7 to 409 per 10^6 bases with increasing UVC dosage from 40 J/m^2 to 1200 J/m^2, while the 6-4PP of TpT increases from 3.7 to 54 per 10^6 bases. The proposed UHPLC-Q-TOF/MS method is promising for accurate identification and quantitative detection of UV-induced dimeric lesions in cellular DNA.

1. Introduction

Solar ultraviolet (UV) radiation is assumed to be a ubiquitously occurring physical carcinogen inducing melanocytic and non-melanocytic skin cancers [1]. UVC and UVB exposure induces mutagenicity and carcinogenesis mainly by the formation of dimeric photoproducts between adjacent pyrimidine bases of genomic DNA. In human skin cells, three major classes of dimeric pyrimidine photoproducts have been identified, including cyclobutane pyrimidine dimers (CPDs), pyrimidine (6-4) pyrimidone photoproduct (6-4PPs), and Dewar valence isomers [2]. Furthermore, it is now well recognized that CPDs are the most frequent UVA-induced DNA lesions in human skin, rather than previously thought 8-oxo-7,8-dihydroguanine (8-OHdG) and DNA strand breaks [3]. These photoproducts can be produced at any of the four dipyrimidine (dC-dC, dC-T, T-dC, and T-T), although with drastically different frequencies [4]. The lesions occur predominantly at dipyrimidine sequences upon excitation of thymine, the most photoreactive base, or, to a lesser extent, of cytosine [5]. The deamination of cytosine-containing CPDs causes a characteristic C to T mutation at TC sites and CC to TT tandem mutations observed in genes such as p53 of UV-induced skin tumors [2]. The characterization and chemical features of 5mC-derived CPDs were also found in DNA fragments [6]. The CPD of thymine-thymine dimer (T < > T) is considered as most abundant and probably most cytotoxic lesions, but the 6-4PP of thymine-thymine dimer (T(6-4)T) has more serious, potentially lethal and mutagenic effects [7]. In contrast, Dewar valence isomers are generated at very low yields in mammalian cells.

UV-induced DNA lesions can influence cell death, aging, mutagenesis and carcinogenesis if they are not completely eliminated by the nuclear DNA repair machinery. Organisms have developed a variety of DNA repair mechanisms in order to counteract those adverse effects of DNA lesions. The major repair pathway for UV lesions is nucleotide excision repair (NER) [9,10] in human cells. Unlike the highly specific photo-reactivation or base excision repair (BER), NER removes a

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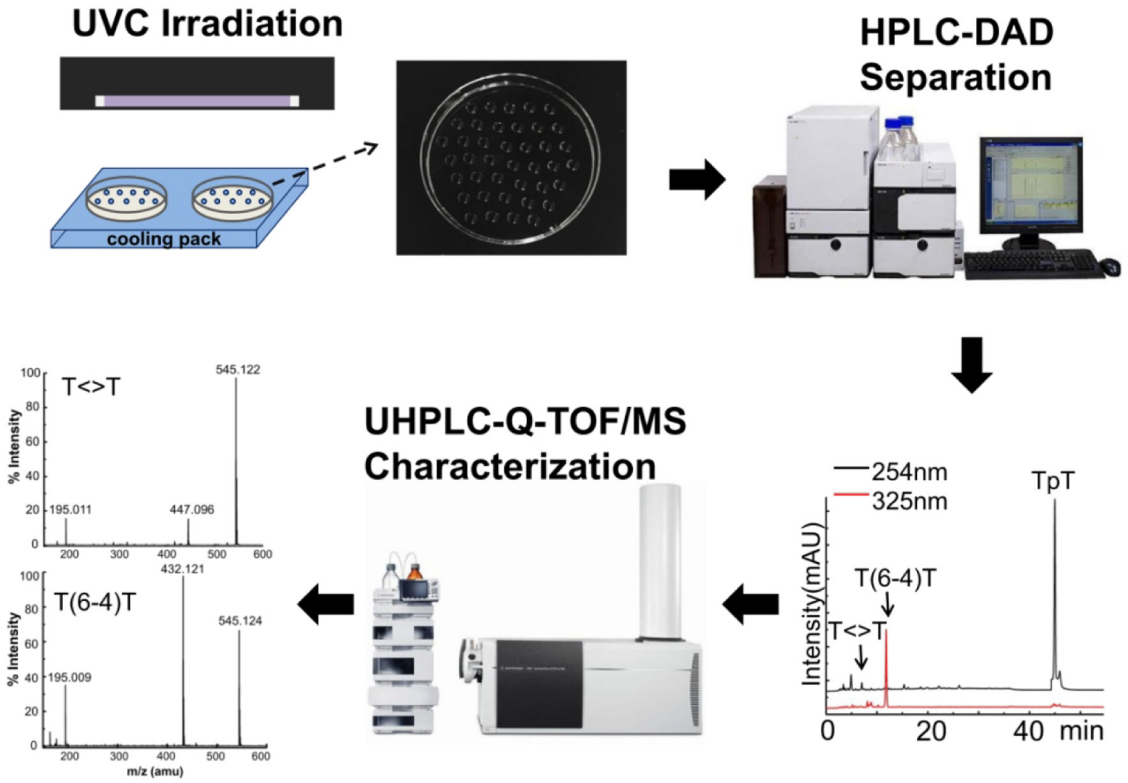

Scheme 1. Procedures for the preparation of T < > T and T(6-4)T dimeric lesions. TT dimer was dispersed in the droplets and irradiated against UVC.

Table 1
Combination of enzyme systems for DNA hydrolysis.

Sample	pH	BSP	SVP	NP1	CIP
A	6.0 (first 2 h)	+	−	+	−
	7.4 (overnight)	+	+	+	+
B	6.0 (overnight)	+	−	+	+
C	6.0 (overnight)	+	+	+	+
D	7.4 (overnight)	−	+	+	+
E	7.4 (overnight)	+	+	+	+

fragment of DNA containing one lesion. Both CPDs and 6-4PPs can be repaired through NER pathway. Interestingly, the methylation at the N6 position of adenosine (m^6A) in RNA occurs within 2 min after the cells exposed to UV radiation [8], suggesting m^6A could be involved in the regulation of DNA repair system for removing UV irradiation-caused damages.

Several methods have been developed for determining UV-induced lesions, including immunological measurements [11–13], electrophoretic techniques [14–16] and chromatographic quantification [17,18]. Immunological methods rely on the production of antibodies raised against UV-irradiated DNA. Those methods were used for the measurement of dimeric pyrimidine photoproducts within the DNA of cultured cells [19] and tissues [20]. A limitation of the immunological approaches is that they suffer from a lack of calibration, so that they only provide relative values for the yields of UV-induced photoproducts, but not absolute values. Electrophoresis-based assays are indirect methods to detect dimeric photoproducts, in which DNA repair enzymes are used to produce additional strand breaks. However, Electrophoresis-based assays usually lack demanded sensitivity and specificity. Chromatographic approaches were also utilized for quantification of photoproducts [21,22]. This approach requires the treatment of DNA with hot acid to release T < > T. However, T(6-4)T could not be analyzed in this assay because of its instability under strong acidic conditions. This limitation was overcome by using hydrofluoric acid (HF) in pyridine as a mild reagent for DNA hydrolysis, followed by HPLC-fluorescence analysis. Unfortunately, HPLC fluorescence based assays are not suitable for CPDs, because they are lack of intrinsic fluorescence. Recently, high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) has been used for the measurement of various of DNA modifications, including 5-hydroxymethyl cytosine (5hmC) [23], 8-oxo-7,8-dihydro-2′-deoxyguanine (8-OHdG) [24], Benzo[*a*]pyrene-7,8-diol-9,10-epxide-dG (BPDE-dG) [25] and others. Douki et al. developed a HPLC-MS/MS method to simultaneously detect T < > T and T(6-4)T, after a two-step enzymatic digestion, in both isolated and cellular DNA exposed to UVC or UVB radiation [17,26]. The accurate and sensitive HPLC-MS/MS method operating in the electrospray ionization (ESI) detection mode provides more quantitative and specific determination of these photoproducts.

In this study, we synthesized and prepared TpT dimeric lesion as standards and developed a one-step enzymatic digestion procedure. Moreover, by the use of the synthetic dimeric lesions as standards and the use of one-step digestion procedure, we established an accurate UHPLC-Q-TOF/MS approach for the detection of thymine-thymine CPD and 6-4PP dimeric lesions in genomic DNA of human cells.

2. Materials and methods

2.1. Materials

The TpT starting materials for generating T < > T and T(6-4)T were synthesized at the BioSune Co., Ltd. (Shanghai, China). Dulbecco's Modified Eagles Medium (DMEM) and penicillin used for cell culture were from GE Healthcare Hyclone (Logan, USA). The fetal bovine serum was from Thermo Fisher Scientific (Waltham, USA). The enzymes for digesting genomic DNA including snake venom phosphodiesterase (SVP), bovine spleen phosphodiesterase (BSP), nuclease P1 (NP1) and calf intestine alkaline phosphatase (CIP) were purchased from Sigma-Aldrich (St. Louis, Mo). HPLC-grade methanol was